

The Effect of Microwave Pulse Duration on the Distance Distribution Function between Spin Labels Obtained by PELDOR Data Analysis

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Abstract. A method is proposed for obtaining a distance distribution function between spin labels in spin-labeled molecules on the basis of the data of pulsed electron-electron double resonance with regard to the finite duration of microwave (mw) pulses. It is shown that taking into account the finite duration of mw pulses makes it possible to extend the range of the studied distances between spin labels to the region of short distance in which the magnitude of the dipole-dipole interaction becomes comparable to or exceeds the spectrum widths of exciting mw pulses. With frozen glassy solutions of biradicals as model systems with a length between spins of less than 2 nm, the shape for the distance distribution function between labels was obtained, and the value and sign of the exchange interaction were estimated. It is demonstrated that the analysis of the dipole-dipole interaction neglecting the duration of mw pulses can lead to substantial distortions in the shape of the distance distribution function.

1 Introduction

The investigation of the dipole-dipole interaction between nitroxyl spin labels in spin-labeled molecules in the solid phase provides information about the distances between labels and allows conclusions about the structure of the molecules studied. When the distances between the labels are less than 1.2 nm, the dipole-dipole interaction between the labels is large enough and can be obtained from the analysis of continuous-wave (CW) electron spin resonance (ESR) spectra or by other methods described in ref. 1. For large distances, the spectra broadening due to the dipole-dipole interaction is hidden under the inhomogeneous spectrum width and cannot be obtained directly from the CW ESR spectra. Weak dipole-dipole interactions can be studied by pulsed methods of ESR spectroscopy on the basis, for example, of electron spin echo (ESE). In this case, the magnitude of the dipole-dipole interaction can be derived whatever the scale of the inhomogeneous spectrum broadening.

Among pulsed ESR methods, the electron-electron double resonance (PELDOR) technique proposed in 1981 [2] for studying weak dipole-dipole interactions between spins in the solid phase appeared to be most effective and was applied for investigations of biradicals [3–6], spin-labeled peptides, and other biomacromolecules [7–9]. The PELDOR technique is based on the study of the behavior of the spin echo signal from one part of the spins (spins A, at the frequency ν_A) during the artificial flip of the other part of the spins induced by a pumping pulse (spins B, at the frequency ν_B). Spins B change their projections for the direction of the external magnetic field under the action of the pumping pulse which causes a change in the sign of the dipole-dipole interaction between spins A and B. As a result, the spin echo amplitude (PELDOR signal) depends on the value of the dipole-dipole interaction between spins A and B, the T scale position of the pumping pulse (Fig. 1), and the probability of spin B flips induced by the pumping pulse. The analysis of these dependences provides the information on the value of the dipole-dipole interaction between spins A and B and, thus, on the distances between spin labels. In the presence of aggregates containing several spin labels, one can estimate the number of labels in aggregates [10–12]. It is worth noting that the dipole-dipole interaction in the PELDOR technique can be accompanied by the exchange interaction between spins with the value J increasing as the distance between spins decreases.

One of the most important recent applications of the PELDOR method is the study of the distance distribution between spin labels rather than fixed distances. In ref. 4 it is shown that the distance distribution function between spin labels in some nitroxide biradicals can be extracted from the analysis of the PELDOR signal decay function when the location of a pumping pulse is changing. In ref. 9, the distance distribution function was derived for glassy solutions of spin-la-

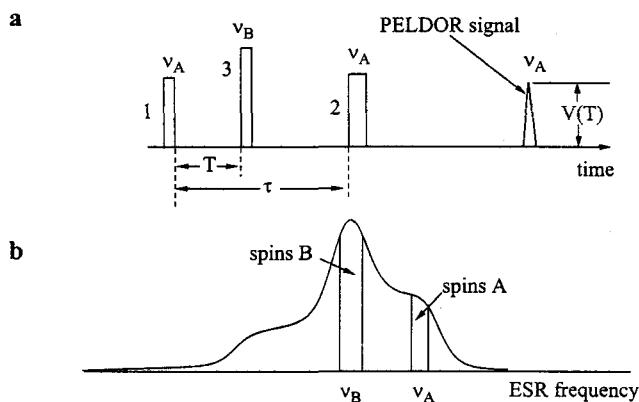


Fig. 1. **a** PELDOR sequence of the microwave (mw) pulses: pulses 1 and 2 at ν_A and the pumping pulse 3 at ν_B . The pulses 1, 2, and 3 display durations, t_{p1} , t_{p2} and t_{p3} , respectively. **b** The ESR spectrum of nitroxyl labels in the solid phase and the arbitrary parts of the ESR spectrum subjected to the effect of the pulses forming echo at the frequency ν_A (spins A) and the pumping pulse at the frequency ν_B (spins B).

beled peptides. The above analysis of the structure of spin-labeled molecules has been performed over a range of distances for which the value of the dipole-dipole interaction between labels is less than the width of the exciting pulses in a frequency domain. In this case, the influence of the duration of mw pulses can be neglected.

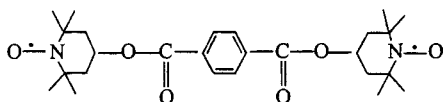
At short distances between labels when the dipolar width ν_{dd} becomes comparable with the spectrum width of the $\pi/2$ mw pulse, $\nu_1 = \pi/(2t_p)$ (t_p is the pulse duration), this problem should be properly taken into account. With $\nu_{dd} = \gamma^2\hbar/r^3$ one can estimate this distance region as $r < 2$ nm for $t_p = 40$ ns. The experimental PELDOR decay in these conditions will have two distinctive features. Firstly, the shift in resonance frequencies of spins A upon the flips of spins B can become so large that they do not take part in the formation of spin echo and therefore give no characteristic dipolar oscillations in the PELDOR signal decay. Moreover, under these conditions, the pumping pulse at ν_B can mainly excite only one of the dipole components of spins B and this will be responsible for the damping of the dipolar oscillations amplitude as compared with the initial PELDOR decay. Secondly, due to the finite duration of the mw pulses, the PELDOR decay at short T times (Fig. 1) will be mainly determined not by dipolar interactions but by the mw pulse duration. This will slow down the PELDOR decay at short T times and in turn will give artificial enhancement of the long-distance part in the distance distribution function obtained from the experimental decay at these conditions. Thus, when analyzing short distances between spin labels, it is necessary to take into account the finite duration of the mw pulses.

The main goal of the present paper is to study the possibility of obtaining the distance distribution function between labels from experimental dependences of PELDOR with regard to the finite duration of mw pulses. In this case, it is of interest to compare the results of the analysis of the distances between the labels with and without regard to the duration of mw pulses. We consider also the possibility of obtaining distance distribution functions along with the value and sign of the exchange interaction from the PELDOR data analysis.

We have studied frozen glassy solutions of nitroxyl biradicals with the structures shown in Fig. 2.

Biradical I has a relatively short chain which connects spin labels. Therefore, we expect a noticeable effect both of the duration of pulses and of the exchange interaction on the PELDOR signal decay. Biradical II was the first biradical for which the PELDOR signal oscillations due to the intramolecular interaction between spin labels was obtained [3]. The distance between spin labels $r = 1.9 \pm 0.16$ nm was measured directly from a period of oscillations assuming that the value of the exchange interaction $|J|$ was less than 2 MHz [3]. In ref. 6 the PELDOR method with a magnetic field jump was employed for biradical II. The distance between labels of 1.94 ± 0.02 nm at the value of the exchange interaction $|J| < 0.1$ MHz was obtained. Note that the estimates given in refs. 3 and 6 were obtained from the PELDOR data neglecting the duration of mw pulses. Thus, it is of interest to determine the distance distribution func-

Biradical I



Biradical II

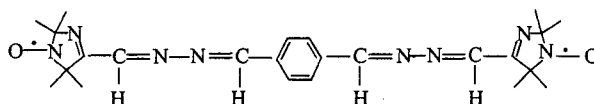


Fig. 2. Structures of biradicals I and II.

tion between spin labels and the value of the exchange interaction for the given biradicals and to compare these data with the previous results.

2 Experimental

Biradicals I and II were synthesized in the Novosibirsk Institute of Organic Chemistry, Russian Academy of Sciences, and donated for investigation by I. A. Grigoryev. PELDOR studies were carried out on a PELDOR spectrometer described in refs. 2 and 13. The duration of the first and second pulses forming the spin echo signal were 40 and 70 ns, respectively. The duration of the pumping pulse was about 40 ns. The position of the pumping pulse corresponded to the maximum amplitude in the ESR spectrum. The frequency difference $\nu_A - \nu_B$ was 65 MHz.

For the PELDOR studies, glassy ampoules with a diameter of 0.5 cm containing about 0.08 ml of the sample solution were used. As a solvent, we used toluene and toluene- D_8 . After freezing at 77 K, this solvent forms transparent glass. The samples were prepared with chemically pure toluene without repurification. The concentration of biradicals varied from $1.5 \cdot 10^{-3}$ to $5 \cdot 10^{-3}$ M. The samples studied were placed in a finger of the Dewar flask cooled with liquid nitrogen and located in the spectrometer resonator.

The CW ESR spectra of the biradicals studied are conventional triplets of nitroxyl radicals in the solid phase.

2.1 Effect of Pulse Duration on the PELDOR Signal Decay

In the present work we used the simplest version of the PELDOR method, i.e., the double-pulse technique of the electron spin echo at the frequency ν_A with the added pumping pulse at the frequency ν_B . A sequence of pulse action is shown in Fig. 1a. Pulses 1 and 2 form an electron spin echo signal at the fre-

frequency ν_A and the pumping pulse 3 induces the flips of spins from another part of the ESR spectrum at the frequency ν_B . The locations of pulses are counted from the back edge of the first pulse. Thereafter, the pulse durations are denoted by t_{p1} , t_{p2} , and t_{p3} for the first, second, and third pulses, respectively. Figure 1b arbitrarily shows the parts of the ESR spectrum subjected to the effect of the pulses forming the echo at the frequency ν_A and the pumping pulse at the frequency ν_B . As mentioned above, the spins participating in the formation of the echo are denoted as spins A, whereas the spins rotated by the pumping pulse are denoted as spins B. The PELDOR signal is the spin echo signal $V(T)$ at a fixed time τ in the presence of the pumping pulse 3.

In ref. 14, the analytical expression was derived for the PELDOR signal of a pair of spin labels with a fixed distance between labels and a given pair orientation with respect to the external magnetic field in the case of rectangular mw pulses of finite duration. The amplitude of the PELDOR signal for the ensemble of these pairs is proportional to the magnetization component M_y and obeys eq. (62) in ref. 14. This equation can be given in a compact form as

$$U(T) = U_1 \sin[D(T + t_{p3}/2)] + U_2 \cos[D(T + t_{p3}/2)] + U_3, \quad (1)$$

$$D = \gamma^2 \hbar (1 - 3\cos^2(\theta)) / r^3 + J. \quad (2)$$

Equations (1) and (2) were derived in ref. 14 for the spin-spin interaction Hamiltonian $\hat{\mathcal{H}} = D\hat{S}_{1z}\hat{S}_{2z}$, where \hat{S}_{1z} and \hat{S}_{2z} are the operators of the spin projections of the first and the second labels on the direction of the external magnetic field, γ is the gyromagnetic ratio for an electron, \hbar is the Planck constant, r is the distance between spin labels in the pair, θ is the angle between the vector connecting spin labels and the magnetic field direction, J is the value of the exchange integral, the value of T is indicated in Fig. 1, t_{p3} is the pumping pulse duration.

The coefficients U_1 , U_2 , and U_3 in Eq. (1) depend on the D value, the resonance frequencies of spin labels, the frequencies, amplitudes and durations of acting mw pulses. For nitroxyl spin labels, the resonance frequencies depend on the hyperfine anisotropy, g tensors, and the orientation of spin labels in a magnetic field. As a result, the coefficients U_1 , U_2 , and U_3 are also dependent on the orientation of spin labels. The specific expressions for these coefficients are given by eq. (62) in ref. 14.

In the limiting case, when pulse durations tend to zero at a constant angle of spin flips and a fairly large difference in the frequencies ν_A and ν_B , Eq. (1) acquires the known form [13]

$$U(T) = U(0)(1 - p_B(1 - \cos(DT))), \quad (3)$$

where p_B is the probability of spin B flips induced by a pumping pulse, $U(0)$ is the PELDOR signal in the absence of a pumping pulse. Equation (3) served as a basis for analyzing the distance between labels and the value of the exchange interaction between the labels in refs. 9, 11 and 13.

In frozen glassy solutions we expect a random orientation of molecules in space. Averaging Eq. (1) over the orientations of biradicals relative to the magnetic field leads to the decay of dipolar oscillations with time T and the tendency of $U(T)$ to its limiting value U_3 . As an example, Fig. 3 shows the $U(T)$ dependence averaged over the angle θ and calculated for a series of the distances r between the labels and pulse durations. The calculations were performed for a random orientation of spin labels at the ends of biradicals. The value of the exchange interaction was taken as zero. In calculations we used the following values for A and g -tensors: $A_{xx} = 4.0$ G, $A_{yy} = 7.0$ G and $A_{zz} = 35.0$ G; $g_{xx} = 2.0095$, $g_{yy} = 2.0061$ and $g_{zz} = 2.0025$. The line shape determined by other sources of the broadening was considered Gaussian, with the width between the points of the maximum slope being 3.9 G. The parameters indicated were used in ref. 9 for determining the distance distribution function in some spin-labeled peptides.

The calculated results are given in Fig. 3 in the coordinates $[\langle U(T) \rangle - \langle U_3 \rangle] / [\langle U_0 \rangle - \langle U_3 \rangle]$ versus T , where U_0 is the $U(T)$ value in the absence of the pumping pulse. The symbol $\langle \dots \rangle$ denotes the averaging over the parameters specified. The U_0 value was calculated from eq. (24) of ref. 14 at the point of the signal maximum.

Figure 3 shows that the mw pulse duration has a considerable effect on the behavior of the PELDOR signal decay. The increase in the pulse duration leads to the decrease in both the amplitude of the oscillation and the value of the PELDOR signal at $T = 0$ with respect to the value of the signal in the absence of the pumping pulse. Of interest is the fact that despite the decrease in the os-

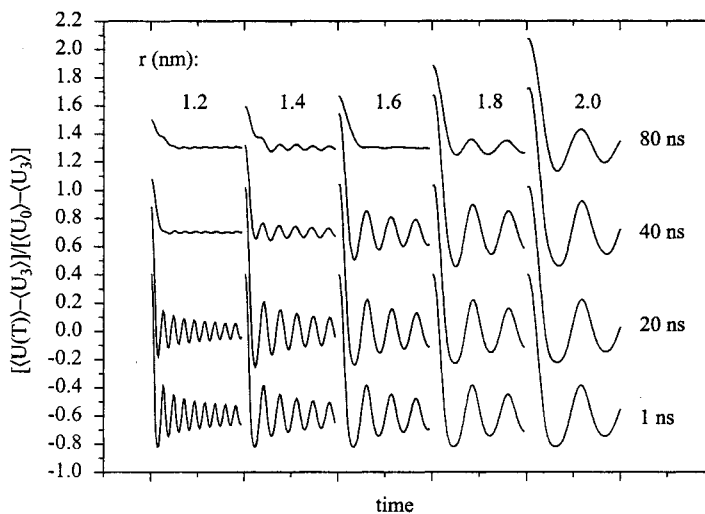


Fig. 3. Calculated PELDOR signal decay for a series of the distances r between the labels and durations of the mw pulses for $t_{p1} = t_{p2} = t_{p3}$. The values of distances and the pulse durations are given in the figure. The rotation angles of the magnetization are equal for all the pulses and amount to 120° .

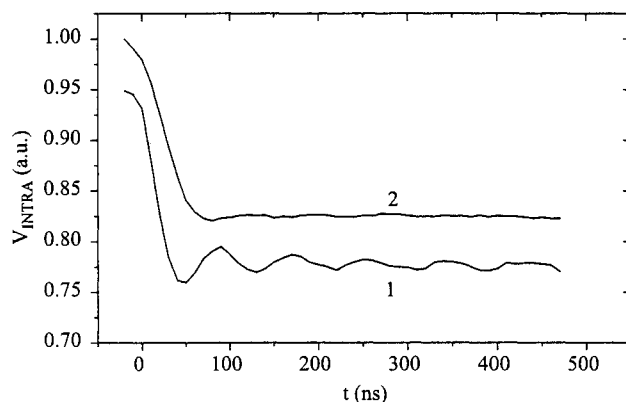


Fig. 4. Effect of the pulse duration on the oscillation amplitude for the solution of biradical I in toluene D_8 . The values of the mw pulse durations were the following: $t_{p1} = t_{p2} = 30$ ns and $t_{p3} = 40$ ns for curve 1, $t_{p1} = t_{p2} = 90$ ns and $t_{p3} = 40$ ns for curve 2. For convenience, curve 1 is shifted down by 0.05.

cillation amplitude, the frequency of dipolar oscillations remains practically constant for a given distance r . This makes it possible to estimate the distance between the labels directly from the period of oscillations whatever the pulse duration. As follows from Fig. 3, for distances of less than 2 nm, this technique is most informative for pulse durations of less than or equal to 20 ns. It is worth noting that for distances exceeding 2 nm, the PELDOR signal amplitude is actually independent of the pulse duration.

Figure 4 presents the experimental PELDOR signal decay due to the intramolecular interaction of spin labels for the frozen glassy solutions of biradical I in toluene- D_8 . As follows from Fig. 4, an increase in the duration of the first and second pulses from 30 to 90 ns leads to the complete disappearance of oscillations. This is in agreement with the general pattern of the behavior of the PELDOR signal (Fig. 3).

It is worth noting that in our experiments, the pulses had not an ideal rectangular shape. The characteristic time of the edge increase and decrease was about 10 ns. The pulses were formed into trapezoids and the pulse width at half-height was considered as the pulse duration.

2.2 Distance Distribution Function with Regard to the Finite Duration of mw Pulses

The aim of the present work is to obtain distance distribution functions between spin labels together with the value of the exchange interaction. In the following the distance distribution function of spin label pairs will be denoted as $F(r) = dn(r)/dr$, where $dn(r)$ is a fraction of pairs in the distance range from r to $r + dr$. The problem of deriving the $F(r)$ form and estimating the J value was solved with Eq. (1). To this end, we used the approach described in ref. 9. It is based

on the calculation of the PELDOR signal amplitudes $\langle U(r_k, T_m) \rangle_{AV}$ for a given set of distances r_k between labels and a set of experimentally used values of T_m . For a given case, the symbol $\langle \dots \rangle_{AV}$ means the averaging of Eq. (1) over all parameters except the distance between the labels and the exchange interaction. The PELDOR signal calculated at each T_m point was represented as the sum of $\langle U(r_k, T_m) \rangle_{AV}$ signals of the pairs with fixed r_k with regard to the fraction of these pairs in the total number of pairs

$$V_{\text{calc}}(T_m) = \sum_k F(r_k) \langle U(r_k, T_m) \rangle_{AV} dr_k,$$

where $V_{\text{calc}}(T_m)$ is the calculated PELDOR signal, $F(r_k)$ is the required value of the distribution function at r_k points; $dr_k = r_{k+1} - r_k$.

To obtain the $F(r_k)$ set, we minimized the sum of the squares of the differences of the experimental $V(T_m)$ and calculated $V_{\text{calc}}(T_m)$ PELDOR signal amplitudes by varying the coefficients $F(r_k)dr_k$ provided that $F(r_k)dr_k \geq 0$,

$$\Delta = \sum_m [V(T_m) - V_{\text{calc}}(T_m)]^2 \rightarrow \min.$$

The solution obtained was minimized by the value of the exchange interaction J . To reduce the manifestation of the instabilities of solution, we have chosen the optimum distance step by increasing the step of dr_k till the Δ value starts to increase. This deteriorates the method's ability for distance resolution but makes it possible to reduce the amplitude of false maxima in the distribution function unavoidable for unstable problems of the given type.

The above approach allows one both to derive the distance distribution function of pairs and to estimate the value and sign of the exchange interaction in biradicals. It was of interest to compare the distribution functions obtained with and without regard to pulse duration. The same approach using Eq. (3) instead of Eq. (1) was used to obtain the distance distribution functions without taking into account the pulse duration.

3 Experimental Data

The information about the interaction between spin labels is contained in the PELDOR signal decay function $V(T)$, where T is the time between the first pulse and the pumping pulse. This decay in general must contain the data on both the intramolecular and intermolecular interactions of spin labels. As a rule, they are mutually independent. Therefore, the experimental signal decay $V(T)$ can be represented as the product of intramolecular V_{INTRA} and intermolecular V_{INTER} contributions to the total dependence $V(T) = V_{\text{INTRA}} V_{\text{INTER}}$. The intramolecular interaction is independent of the biradical concentration and can be separated from intermolecular interactions by studying the concentration dependences of the PELDOR signal decay. As in refs. 3 and 15, this method was used in the

present work to distinguish the intramolecular interaction of labels in the biradicals studied.

Biradical I. Figure 5 shows experimental and calculated $V(T)_{\text{INTRA}}$ for biradical I. The solid lines were calculated with the distribution function shown in Fig. 6. As follows from Fig. 5, the oscillation beats are observed for biradical I indicating the existence of two similar oscillation frequencies. It is worth noting that the oscillation frequency is close, in this case, to the Larmor proton frequency in the working magnetic field. The dependences of V_{INTRA} on T for solutions of biradical I in fully deuterated toluene were obtained to verify a possible effect of solvent protons on the behavior of the PELDOR signal decay. The decays obtained for both solvents actually coincide. Thus, the oscillations shown in Fig. 5 are determined by the dipolar intramolecular interaction of spin labels rather than by the interaction of unpaired electrons with solvent protons.

Figure 6 shows the distance distribution functions between labels obtained by two methods. Curve 1 was derived by taking into account experimental mw pulse durations (Eq. (1)). Curve 2 was obtained for the infinitely short pulses from Eq. (3). The value of the exchange interaction between the labels obtained for both cases is $J = 1.4 \pm 0.5$ MHz. Note that despite the substantial difference in the shape of distance distribution functions in Fig. 6, both functions provide the same fair description of the experimental data presented in Fig. 5.

Figure 6 shows that, unlike curve 2, the distribution function 1 contains two maxima at distances of 1.62 and 1.7 nm. The total width of the distribution function 1 at half-height is 0.16 nm. The uncertainty of the obtained J value leads

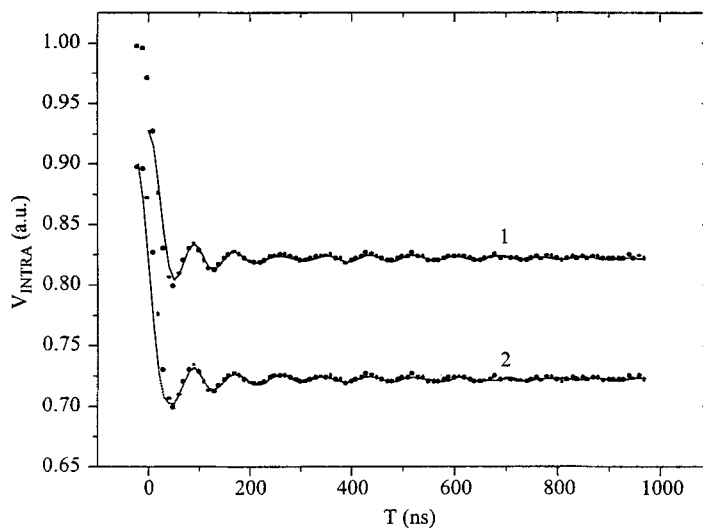


Fig. 5. PELDOR signal decay $V_{\text{INTRA}}(T)$ for the frozen glassy solution of biradical I in toluene at 77 K. The dots denote the experimental data, the solid lines were calculated with the distribution functions given in Fig. 6. Curve 1 was calculated with the distribution function 1 in Fig. 6, curve 2 was calculated with the distribution function 2 in Fig. 6. For convenience, curve 2 is shifted down by 0.1.

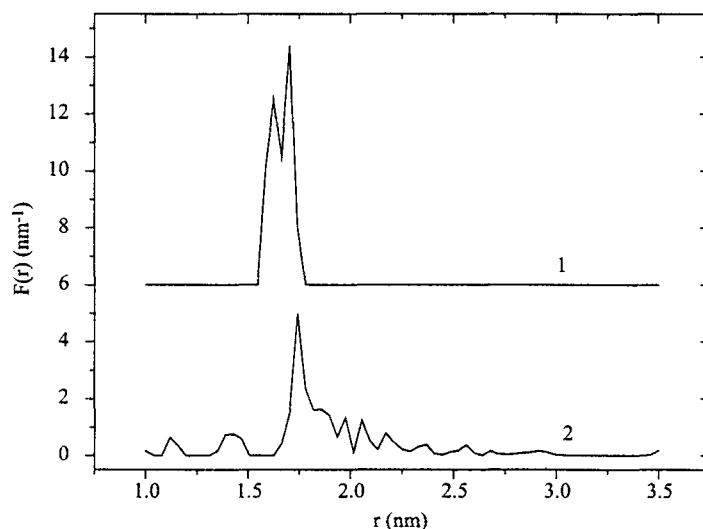


Fig. 6. Distance distribution function $F(r)$ between spin labels for biradical I in toluene at 77 K. Curve 1 was calculated with regard to the mw pulse duration, curve 2 was calculated without regard to the pulse duration. For convenience, curve 1 is shifted up by 6 nm^{-1} .

to the shift in the distribution function by $\pm 0.04 \text{ nm}$ without a noticeable change in the $F(r)$ shape.

Biradical II. Figure 7 shows the experimental and calculated dependences of $V(T)_{\text{INTRA}}$ for biradical II. The solid lines were calculated with the distribution functions presented in Fig. 8. Curve 1 was obtained by taking into account the mw pulse durations and belongs to the distribution function 1 in Fig. 7. Curve 2 was obtained without regard to the pulse duration on the basis of Eq. (3) and belongs to the distribution function 2 in Fig. 8.

Figure 8 shows the distance distribution functions between labels obtained by two methods. Curve 1 was obtained by taking into account the experimental mw pulse durations (Eq. (1)). The distribution function maximum is reached, in this case, for a distance of 1.99 nm . The width of the distribution function at half-height is 0.06 nm . Curve 2 was obtained for the infinitely short pulses (Eq. (3)). The maximum of curve 2 is at the same distance as that for curve 1. The best agreement between experimental and calculated curves was obtained for the value of the exchange interaction between labels $J = 0.7 \pm 0.4 \text{ MHz}$. The uncertainty in the determination of the J value causes a shift in the distribution function maximum by $\pm 0.03 \text{ nm}$.

The form of the distribution function 1 in Fig. 6 for biradical I indicates that in this case there are at least two conformations corresponding to distances of 1.62 and 1.7 nm between labels. The obtained value $J = 1.4 \text{ MHz}$ is likely to be averaged over two conformations. It is possible that the exchange interaction occurring in the molecular frame of the biradical and depending on the number

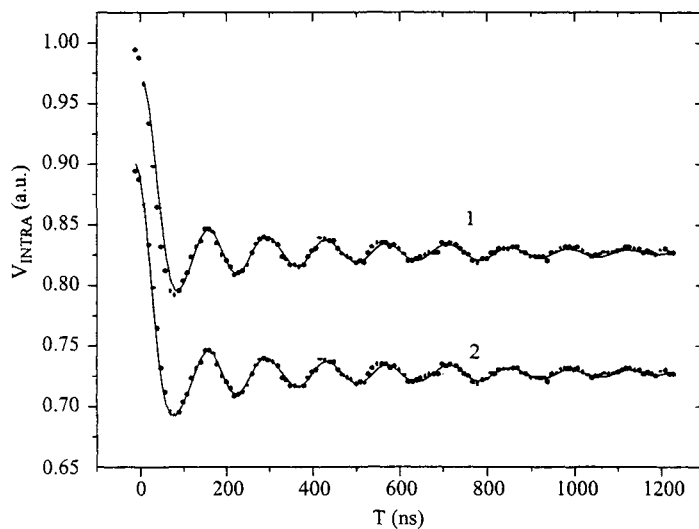


Fig. 7. PELDOR signal decay $V_{\text{INTRA}}(T)$ for the frozen glassy solution of biradical II in toluene at 77 K. The dots denote the experimental data, the solid lines were calculated by the distribution functions shown in Fig. 8. Curve 1 was calculated with the distribution function 1 in Fig. 8, curve 2 was calculated with the distribution function 2 in Fig. 8. For convenience, curve 2 is shifted down by 0.1.

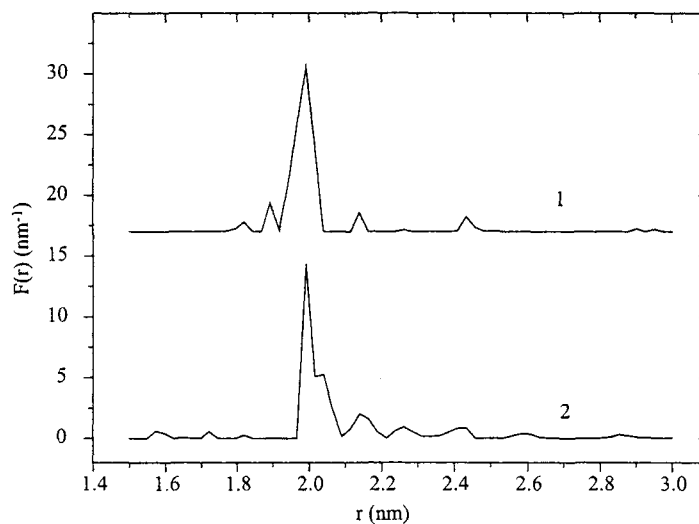


Fig. 8. Distance distribution function $F(r)$ between spin labels for biradical II in toluene at 77 K. Curve 1 was obtained with regard to the mw pulse duration, curve 2 was obtained without regard to the pulse duration. For convenience, curve 1 is shifted up by 17 nm^{-1} .

and type of bonds may be the same in both conformations. Of interest is the fact that the value of the exchange interaction is substantially smaller than the constant of the dipole-dipole interaction of 14 MHz. It should be mentioned that we received positive values of the exchange interaction for both biradicals.

The maximum of the distribution function 1 in Fig. 8 for biradical II is at a distance of 1.99 ± 0.03 nm, which is close to the previous value obtained for the distance between spin labels in refs. 3 and 6 by assuming the absence of the distance distribution between labels. Note that the value $J = 0.7$ MHz obtained in the present work exceeds the value of 0.1 MHz obtained in ref. 6 as the upper boundary for J . This discrepancy is probably due to the difference in the models used to calculate these parameters.

Figures 6 and 8 show that distribution functions 2 obtained for both of the biradicals for infinitely short pulses differ substantially from curves 1 in the same figures. The comparison of dependences 1 and 2 for each case indicates that the use of Eq. (3) in the analysis of short distances between spin labels can lead to substantial distortions in the resulting distance distribution functions. The main important difference is the occurrence of the long distances in the distance distribution functions 2 compared with 1. As described above, these features are connected with the slowing-down effect on the PELDOR decay at short T times due to the finite duration of the mw pulses. It is seen that as the distance between labels increases, i.e., upon transition from biradical I to biradical II, the difference in distribution functions 1 and 2 becomes much smaller.

Obviously, the use of equations derived in ref. 14 for the rectangular shape of mw pulses for describing the behavior of the PELDOR signal is approximate and not always justified under experimental conditions. From Fig. 3 it follows that the most effective way of studying short distances between labels is the application of pulses with duration of not longer 20 ns. However, the rectangular pulses of this duration can be obtained with resonators of a fairly low Q factor and powerful sources of mw pulses. Therefore, it is necessary to describe theoretically the behavior of the PELDOR signal decay for the real form of the exciting mw pulses.

4 Conclusions

A method is proposed for obtaining the distance distribution function between spin labels in biradicals in the solid phase by the data of PELDOR spectroscopy on the dipole-dipole interaction between labels with regard to the finite duration of mw pulses. It is shown that taking into account the finite duration of mw pulses allows one to extend the range of the studied distances between spin labels to short distances within which the value of the dipole-dipole interaction between labels becomes comparable with or exceeds the spectrum width of the exciting mw pulses.

For the frozen glassy solutions of biradicals with different chain lengths between labels, we obtained the form of the distance distribution function and the

value of the exchange interaction between labels. It is shown that the analysis of the dipole-dipole interaction without regard for the duration of mw pulses can cause substantial distortions in the distance distribution function which increases with decreasing distance between labels.

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References

1. Berliner L.J., Eaton S.S., Eaton G.R. (eds.): *Biological Magnetic Resonance*, vol. 19, chapt. 1. New York: Kluwer Academic/Plenum 2000.
2. Milov A.D., Salikhov K.M., Schirov M.D.: *Fiz. Tverd. Tela (Leningrad)* **23**, 975 (1981)
3. Milov A.D., Ponomarev A.B., Tsvetkov Yu.D.: *Zh. Strukt. Khim.* **25**, 51 (1984)
4. Jeschke G., Koch A., Jonas U., Godt A.: *J. Magn. Reson.* **155**, 72 (2002)
5. Weber A., Schiemann O., Bode B., Prisner T.F.: *J. Magn. Reson.* **157**, 277 (2002)
6. Kulik L.V., Grishin Yu.A., Dzuba S.A., Grigoryev I.A., Klyatskaya S.V., Vasilevsky S.F., Tsvetkov Yu.D.: *J. Magn. Reson.* **157**, 61 (2002)
7. Bernati M., Weber A., Antonic J., Perjstein D.L., Rabblee J., Stubbe J.: *J. Am. Chem. Soc.* **125**, 14988 (2003)
8. Schiemann O., Weber A., Edwards T.E., Prisner T.F., Sigurdson S.: *J. Am. Chem. Soc.* **125**, 3434 (2003)
9. Milov A.D., Tsvetkov Yu.D., Formaggio F., Oancea S., Toniolo C., Raap J.: *J. Phys. Chem. B* **107**, 13719 (2003)
10. Milov A.D., Ponomarev A.B., Tsvetkov Yu.D.: *Chem. Phys. Lett.* **110**, 67 (1984)
11. Milov A.D., Tsvetkov Yu.D., Formaggio F., Crisma M., Toniolo C., Raap J.: *J. Am. Chem. Soc.* **122**, 3843 (2000)
12. Jeschke G.: *Macromol. Rapid Commun.* **23**, 227 (2002)
13. Milov A.D., Maryasov A.G., Tsvetkov Yu.D.: *Appl. Magn. Reson.* **15**, 107 (1998)
14. Maryasov A.G., Tsvetkov Yu.D.: *Appl. Magn. Reson.* **18**, 583 (2000)
15. Milov A.D., Tsvetkov Yu.D., Formaggio F., Crisma M., Toniolo C., Raap J.: *J. Peptide Sci.* **9**, 690 (2003)

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